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New environmentally friendly microbiological composition for comprehensive protection of wheat from diseases and pests

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The multifunctional biopreparation (MB) containing bacterial cells of *Bacillus subtil*is 26D (ARRIAM 128), *Bacillus thuringiensis* ssp. *thuringiensis* (VKPM B-5689) and *Bacillus thuringiensis* ssp. *kurstaki* (VKPM B-6066) for increasing crop yields and protect them from pests and diseases was developed. Assessment of protective and growth-stimulating properties of MB in the field revealed that two-time treatment of spring wheat plants Vatan variety with the 2 kg/ha MB allowed obtaining the maximum increase of harvest. It amounted to 0.48 ton/ha of grain in comparison with the control (water treatment). The efficiency of MB application for protection of wheat against aphids was 100%. The use of a single and double concentration of mixture of biofungicide Phytosporin-M (*B. subtilis* 26D, $2 \cdot 10^9$ CFU/g, 1 kg/ha and 2 kg/ha) and bioinsecticide Bitoxybacillin (1 kg/ha and 2 kg/ha) did not allow to obtain an effect equal to MB influence. The developed environmentally safe microbiological consortium was recommended as the basis of biopreparation with multifunctional activity.

Keywords: microbiological preparation, *Bacillus* sp., wheat protection.

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Новая экологически безопасная микробиологическая композиция для комплексной защиты пшеницы от болезней и вредителей

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Испытана эффективность применения нового многофункционального биопрепарата, содержащего клетки бактерий *Bacillus subtilis* 26Д (ВНИИСХМ 128), *Bacillus thuringiensis* ssp. *thuringiensis* (ВКПМ B-5689) и *Bacillus thuringiensis* ssp. *kurstaki* (ВКПМ B-6066), для защиты посевов пшеницы от насекомых-вредителей, болезней и увеличения урожайности зерна. Двукратная обработка яровой пшеницы сорта Ватан биопрепаратом позволила получить в эксперименте максимальную прибавку урожайности зерна в 0,48 т/га в сравнении с контролем (обработка водой). Эффективность защиты от злаковой тли составила 100%. Многофункциональный биопрепарат рекомендован в качестве основы экологически безопасного биопестицида для применения на посевах пшеницы.

Ключевые слова: микробиологический препарат, *Bacillus* sp., защита пшеницы.

In order to reduce the frequency of treatments of crops with chemical plant protection products, some companies have developed a number of chemical combined agents (insectofungicides), for example, such as Celest Top (Syngenta), Prestige, Emesto Quantum (Bayer) and others. Their effectiveness is quite high, but the problems of the formation of resistance of harmful organisms, the accumulation of chemicals in the environment, and toxicity to animals and humans remain [1]. Therefore, it is of interest to develop safe biological plant protection products with multifunctional activity, combining simultaneously fungicidal, insecticidal and plant growth stimulating properties. The presence of combined activity in biological products can be achieved through the creation of multicomponent formulations, including several strains of microorganisms with various economically useful properties. Currently, there are known microbial preparations with fungicidal activity based on the bacteria *Bacillus subtilis*, *B. amyloliquefaciens*, for example, Fitosporin-M (BashInkom LLC), Alirin-B (Agrobiotechnology LLC), Orgamika S (Bionovatic LLC) [2], as well as biological products for the control of insect pests based on *B. thuringiensis* (*B. th.*), for example, Bitoxibacillin (Sibbiopharm LLC), containing spores and cells of the *B. th.* ssp. *thuringiensis* 98 ($BtH_1$98) and δ-exotoxin [3]. The presence of different economically useful properties in bacteria of the same genus *Bacillus*, their ability to sporulate and produce dry preparations on their basis, makes it possible to classify bacteria of this genus as promising for use as a basis for polyfunctional plant protection products. Confirmation is, for example, patent EP2061326A1 [4], in which a consortium of strains of wild, mutant or genetically modified forms of bacteria *Bacillus* spp. is used to protect plants from various harmful organisms.

Treatment of plants with biological products based on rhizosphere or phylloplanic bacteria leads to their gradual elimination due to the competitive pressure of native microorganisms. One of the solutions to exclude their competitive pressure can be the use of endophytic bacteria that can live inside plants, not only without harming them, but also protecting the host. It is known that some "fungicidal" strains of bacteria *Bacillus* spp., as well as "insecticidal" *B. th.* [5, 6] have endophytic properties. However, the number of plant protection products based on endophytes is limited. In this regard, we have proposed a multifunctional preparation containing endophytic strains of bacteria of the genus *Bacillus*. The results of evaluating the effectiveness of its use for protecting wheat from pests and diseases are given in this work.

The aim of the work is to evaluate the effectiveness of using a new preparation containing cells *Bacillus subtilis* 26D (ARRIAM 128), *Bacillus thuringiensis* ssp. *thuringiensis* (VKPM B-5689) and *Bacillus thuringiensis* ssp. *kurstaki* (VKPM B-6066) for yield increase and protection of wheat from insect pests and diseases.

Research methods

Strains of bacteria *B. th.* ssp. *thuringiensis* that are not pathogenic for humans and animals (number in the VKPM collection V-5689) and *B. th.* ssp. *kurstaki* (VKPM V-6066), as well as *B. subtilis* 26D (collection of ARRIAM, No. 128), were provided by BashInkom LLC. Bacterial preparations were obtained separately by cultivating them in liquid L-medium according to Miller (DIFCO) until sporulation. Then the spores were separated from the medium by centrifugation, mixed with a starch-sucrose mixture (1 : 1 by weight) and dried in an air flow in a laminar-flow cabinet. The preparations containing at least 4 billion colony-forming units of bacteria (CFU) in 1 g of each powder have been obtained. The powders were mixed in such a ratio by weight so that 1 g of the mixture contained $2 \cdot 10^9$ CFU of *B. subtilis* 26D bacteria, $1 \cdot 10^9$ CFU of *B. th.* ssp. *thuringiensis* and $1 \cdot 10^9$ CFU of *B. th.* ssp. *kurstaki*.

To compare the effectiveness of MB, a mixture of dry commercial preparations of the biofungicide Fitosporin-M (*B. subtilis* 26D, $2 \cdot 10^9$ CFU/g, BashInkom LLC) and the insecticide Bitoxibacillin (BTB) (*B. th.* ssp. *thuringiensis* $H_1$98, $20 \cdot 10^9$ CFU/g, Sibbiopharm LLC) has been prepared.

The effectiveness of the preparations was assessed in the fields of the educational and scientific center of the Bashkir State Agrarian University according to the methods of [7] and the All-Russian Research Institute of Plant Protection of the Russian Academy of Agricultural Sciences [8]. Repetition was three times, plot area 50 m^2.

The biopreparations were suspended in water, the crops were treated with a Zhuk knapsack sprayer at the rate of 300 L of working liquid per hectare (Table 1).

The results were processed using the Statistica 12.0 software. The tables show the mean values of biological repeats and their standard deviations or the values of the least significant difference with the control at $p = 0.05$.

Table 1

Scheme of plants treatment

Variants of treatment	Preparations doses for one treatment, kg/ha	Tillering	Full blossom
1	Control, water 300 L/ha	+	+
2	Phytosporin, 1 kg/ha + BTB 1 kg/ha	+	–
3		+	+
4	MB 2 kg/ha	+	–
5		+	+

Note: "+" plants treated; "–" plants no treated.

Results and discussion

The manifestation of physiological activity by endophytes, being inside plant tissues, makes it possible to reduce the number of plant treatments due to long-term effective mutualism of the microorganism and the host. At the same time, the microsymbiont is protected from the influence of the environment by plant tissues, and its presence in the agroecosystem is limited by the host (9). Therefore, the use of preparations based on endophytes increases the likelihood of the effectiveness of plant protection from harmful organisms.

Treatment of wheat crops in the tillering phase with a mixture of commercial preparations slightly stimulated plant growth up to the earing phase. The effect of the MP in variant 4 was manifested in the stimulation of plant growth in the heading phase, and in variant 5 – in the phase of grain waxy ripeness.

The use of biological products decreased the prevalence of powdery mildew, mixed root rot and septoria blight, as well as plant damage by leaf rust. Double treatment of crops with a mixture of commercial preparations, in comparison with MB, was more effective in protecting plants from leaf rust (Table 2). Single and double treatment of plants with MB effectively protected plants from powdery mildew and root rot in comparison with treatment with a mixture of commercial preparations. The crops of wheat treated with MB did not contain cereal aphids (Table 2), which may be associated with a combination of production of entomotoxins by *B. th.* and the synthesis of surfactin by *B. subtilis* strain 26D, which, as previously reported, is characterized by aficidity [10]. The negative effect of bacteria on the vital activity of aphids could also be associated with an increase in plant tolerance to damage [11], probably due to the production of phytohormones by microorganisms that stimulate plant growth, increase the rate of photosynthesis, as well as the availability of mineral nutrition elements [11, 12].

On the plots treated with MB, the number of leafhoppers decreased by almost 2 times in comparison with the control plots. Thrips also turned out to be sensitive to the components of the preparation. The least protective effect of MB was observed in relation to bread beetles and cereal flies.

Stimulation of plant growth by the MB with simultaneous protection against damage by insects and phytopathogens contributed to the formation of greater biological productivity. With a single treatment of plants with the MB, the grain yield in comparison with a single action of the mixture of Fitosporin-M and BTB increased by 0.26 ton/ha, and with a double application – up to 0.39 ton/ha in comparison with a double treatment with commercial preparations (Table 3). In general, one-

Table 2

Effect of treatment of wheat plants with biopreparations on phytopathological condition and on propagation of pests

Variant*	Prevalence rate of diseases, %				Insect pests (specimens per corresponding number of net swings at "milk earing phase" phase of wheat grain)				
	powdery mildew (*Erysiphe graminis*) at earing phase	septoria at "milk earing" phase	brown rust at "milk earing" phase	common root rot	greenbug	leafhoppers	thrips	cereal fleas	cereal flies
1	20	96	84	52	58±7	38±4	296±7	703±12	61±3
2	12	88	76	49	44±5	38±4	160±10	664±16	50±7
3	12	80	44	46	53±3	34±2	154±7	615±16	44±2
4	2	84	76	44	not found	20±1	216±12	610±12	52±5
5	2	76	72	42		20±1	194±9	601±7	54±4

Note: Variants of treatment see in Table 1.*

Table 3

Effect of biopreparation on growth and productivity of soft wheat plants

Variant*	Plants height, sm		Number of stems, things/m^2	Ear length, cm	Number of grains in ears, things	Weight of grains in one ear, g	Yield, ton/ha
	earing phase	phase of wax ripeness					
1	52.3	65.2	232	6.7	21	0.668	1.55
2	53.9	65.7	247	6.2	21	0.660	1.63
3	53.2	67.2	251	6.5	21	0.653	1.64
4	55.3	72.4	249	7.4	24	0.759	1.89
5	54.8	73.7	251	7.4	26	0.809	2.03
** $LSD_{0.05}$	2.5	3.3	11	1.0	3	0.036	0.11

*Note: * Variants of treatment see in Table1; ** Least significant difference at 0.05 significance level.*

and two-fold application of MB with a rate of 2 kg/ha allowed to obtain the largest increase in grain yield in the experiment, respectively, 0.34 and 0.48 ton/ha in comparison with the productivity on the control plots.

Conclusion

Thus, it was found that the treatment of wheat plants with a new ecologically safe microbiological composition effectively suppresses the spread of major diseases and harmful insects on the crops of these crops and allows a large increase in grain yield in comparison with the use of a mixture of commercial preparations BTB and Fitosporin-M. The use of the new microbiological composition allowed to obtain an additional 0.32 ton/ha of grain in comparison with the use of the preparations of combination of Fitosporin-M and BTB.

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